AVIAN DISEASES 50:430–433, 2006

Research Note—

Presence of Inoculated *Campylobacter* and *Salmonella* in Unabsorbed Yolks of Male Breeders Raised as Broilers

N. A. Cox,[A] L. J. Richardson,[A] R. J. Buhr,[A] J. K. Northcutt,[A] B. D. Fairchild,[B] and J. M. Mauldin[B]

[A]Russell Research Center, USDA-ARS, Athens, GA
[B]Department of Poultry Science, University of Georgia, Athens, GA

Received 2 December 2005; Acepted 25 February 2006

SUMMARY. Day-old male broiler breeder chicks were obtained from a commercial hatchery and raised as broilers. For Experiment 1, at 5 wk of age, the broilers were orally inoculated with a 10^6cfu/ml of a characterized strain of *Campylobacter jejuni* and a cocktail (three naladixic acid-resistant strains) of *Salmonella* serovars. One week after inoculation, the birds were euthanatized and defeathered. The abdominal cavity was examined and any unabsorbed yolk material (and remaining yolk stalk) and ceca were aseptically removed for microbiological analyses. For each pooled sample (two birds per pool), an aerobic plate count (APC), an *Enterobacteriaceae* (ENT) count, and a test for the presence of *Campylobacter* and *Salmonella* was performed. For Experiment 2, at 5 wk of age, the broilers were orally inoculated with 10^5cfu/ml of a characterized strain of *Campylobacter jejuni*. One week after inoculation, the birds ($n = 20$) were killed, defeathered, and the yolk stalk, attached yolk, or free-floating yolk and ceca were individually analyzed for presence of *Campylobacter*. For Experiment 1, the *Salmonella*-inoculated birds had 2/12 ceca and 0/12 unabsorbed yolk samples positive for *Salmonella*. The average yolk APC was $\log_{10}$ 3.4 cfu/g and the average ENT was $\log_{10}$ 1.9 cfu/g. For the *Campylobacter*-inoculated birds, 12/12 ceca and 9/12 unabsorbed yolk samples were positive for *Campylobacter*. The average yolk APC was $\log_{10}$ 3.5 cfu/g and the average ENT was $\log_{10}$ 3.1 cfu/g. For Experiment 2, the inoculated *Campylobacter* birds had 19/20 ceca, 5/20 free floating yolks, and 19/20 yolk stalks positive. In Experiment 1, the inoculated *Campylobacter* colonized the ceca in every instance and were present in 75% of the unabsorbed yolks. Alternatively, the inoculated *Salmonella* were not found in any of the unabsorbed yolks and only rarely in the ceca. In Experiment 2, the inoculated *Campylobacter* was found in very high numbers in the yolk and internal body samples. Determining to what extent these internal bodies and unabsorbed yolks play in bacterial colonization and contamination of the birds at processing has not been determined. The next step will be to determine the incidence of unabsorbed yolks and presence of *Campylobacter* and *Salmonella* in these bodies of commercial broilers at processing.

RESUMEN. *Nota de Investigación*—Presencia de *Campylobacter* y *Salmonella* inoculados en saco vitelino no reabsorbido de reproductores machos criados como pollos de engorde.

Pollitos reproductores de engorde de un día de edad se obtuvieron de una incubadora comercial y fueron criados como pollos de engorde. Para el experimento 1, los pollos fueron inoculados a las cinco semanas de edad por vía oral con 10^6 unidades formadoras de colonia (UFC) por ml de una cepa caracterizada de *Campylobacter jejuni* y una mezcla de tres serovares de *Salmonella* resistentes al ácido nalidíxico. Las aves fueron sacrificadas y desplumadas una semana después de la inoculación. Se examinó la cavidad abdominal y se removió asépticamente el saco vitelino y cualquier material no reabsorbido del mismo, así como los ciegos, con la finalidad de realizar análisis microbiológicos. Para cada uno de los grupos de muestras (dos aves por muestra) se realizó un recuento de aerobios, un recuento de enterobacterias y una prueba para determinar la presencia de *Campylobacter* y *Salmonella*. Para el experimento 2, las aves fueron inoculadas a las cinco semanas de edad por vía oral con 10^5 UFC /ml de una cepa caracterizada de *Campylobacter jejuni*. Las aves ($n = 20$) fueron sacrificadas una semana después de la inoculación y el saco vitelino y los ciegos se analizaron individualmente para determinar la presencia de *Campylobacter*. Para el experimento 1, en las aves inoculadas con *Salmonella* dos de 12 ciegos y ninguno de los 12 sacos vitelinos resultó positivo a *Salmonella*. El promedio del recuento de aerobios fue equivalente al logaritmo$_{10}$ 3.4 UFC/gramo y el promedio del recuento de enterobacterias fue de logaritmo$_{10}$ 1.9 UFC/gramo. En las aves inoculadas con *Campylobacter*, todos los ciegos (12 de 12) y nueve de 12 vitelos no reabsorbidos resultaron positivos para *Campylobacter*. El promedio del recuento de aerobios fue de logaritmo$_{10}$ 3.5 unidades UFC/gramo y el promedio del recuento de enterobacterias fue de logaritmo$_{10}$ 3.1 UFC/gramo. Para el experimento 2 las aves inoculadas con *Campylobacter* mostraron 19 de 20 ciegos, cinco de 10 vitelos libres y 19 de 20 sacos vitelinos positivos. En el experimento 1 el *Campylobacter* inoculado colonizó el ciego en cada oportunidad y se encontró en el 75% de los vitelos no reabsorbidos. Por el contrario, la *Salmonella* inoculada no se encontró en ninguno de los vitelos no reabsorbidos y solo de forma esporádica en el ciego. En el experimento 2 el *Campylobacter* inoculado se encontró en un número muy alto de los vitelos y en muestras de cuerpos internos. No se ha determinado hasta que punto estos vitelos no reabsorbidos y estos cuerpos internos juegan un papel en la colonización bacteriana y la contaminación de las aves en la planta de procesamiento. El próximo paso será determinar la incidencia de los vitelos no reabsorbidos y la presencia de *Campylobacter* y *Salmonella* en estos cuerpos en los pollos de engorde comerciales al momento del procesamiento.

Key words: broiler, *Campylobacter*, *Salmonella*, *Enterobacteriaceae*, aerobic plate count, unabsorbed yolk, and ceca

Abbreviations: APC = aerobic plate count; cfu = colony forming unit; ENT = *Enterobacteriaceae*; TPC = total plate count; VRBG = violet bile red glucose agar

Campylobacter and *Salmonella* contaminations are a major concern to the poultry industry due to the organisms being recognized as causes of acute bacterial gastroenteritis in humans (22). Epidemiologic evidence has implicated poultry products as a significant source of these human infections in the United States and other developed countries (7,17). The infection of these foodborne bacteria in humans has been closely associated with the consumption of undercooked poultry or consumption of foods cross-contaminated from raw poultry products (9,17). The Centers for Disease Control and Prevention estimates that, in the United States, human cam-

pylobacteriosis infection accounts for more than 2.4 million cases of gastroenteritis annually, and 80% of the cases are considered foodborne (12). Olsen *et al.* (17) found that *Salmonella* account for the majority of gastroenteritis outbreaks in the United States and the consumption of poultry has been implicated in 40% of the cases. *Campylobacter jejuni*, one of the most common species carried in the alimentary tract of poultry in levels of up to 10^8 cfu/g of feces content can be isolated frequently from the ceca of poultry (8). This accounts for the high incidence of *C. jejuni* in poultry processing plants and on processed carcasses due to contamination from the digestive tract contents (18). The United States Department of Agriculture Food Safety and Inspection Service baseline studies of foodborne pathogens on broiler carcasses discovered that approximately 88% of fresh broiler carcasses at the market were contaminated with *C. jejuni* (23). *Salmonella* contamination is also found readily on fresh broiler carcasses at market, but not in as high an incidence as *Campylobacter*. In a study by Simmons *et al.* (21) they found that approximately 34% of fresh carcasses at market were contaminated with *Salmonella* (21).

Recently, it has been observed that numerous broilers in the processing plant contain what is commonly referred to as unabsorbed yolk contents. These yolk contents can be attached to the Meckel's diverticulum or can be free floating inside the abdominal cavity of the bird. If human pathogenic bacteria that is known to be associated with poultry can thrive in these unabsorbed yolks during the grow-out stage of broilers, these unabsorbed yolks could be a point of recolonization of these broilers in grow-out facilities. In addition, these unabsorbed yolks could be a possible mode of contamination in processing plants and on processed broilers.

In a developing avian embryo, the yolk is utilized for energy (19). The yolk can compose 20% of the body weight of newly hatched chicks. After chicks hatch and have access to feed and water, they undergo metabolic adaptations while moving from embryonic yolk dependence to utilization of exogenous feed. At this same period, you see a gradual absorption of the yolk into the chick's digestive system (16). Chicks normally forage and ingest feed following hatch, and growth commences approximately 24 hr after initiation of feed intake (6). However, under practical conditions, chicks may not receive feed until 36–48 hr after hatching. Therefore, numerous studies have been conducted in determining the effects of feed and water deprivation on yolk absorption and body-weight gain over the course of a grow out (1,3,10,13,14,15).

However, in certain instances, the yolk does not become completely absorbed. When this happens, the unabsorbed yolk can remain attached to the Meckel's diverticulum (yolk stalk), or the unabsorbed yolk can become detached and be free floating in the peritoneal cavity. The Meckel's diverticulum is connected to the small intestines and is located on the antimesenteric side at the jejunoileal junction on the distal half of the small intestines. Numerous studies have shown that *Salmonella* can be recovered from the yolk sac contents of young chicks (11,20). However, the recovery or examination of bacterial contamination and its significance in broilers or breeders has not been investigated to our knowledge. The objective of this study was to determine if inoculated *C. jejuni* and *Salmonella* serovars could be detected in unabsorbed yolks of broilers at the end of their grow out and determine the levels of bacteria in these unabsorbed yolks.

MATERIALS AND METHODS

Experimental design. For Experiments 1 and 2, male breeder chicks ($n = 300$) were obtained from a commercial hatchery at day of age and placed in experimental grow-out facilities at the University of Georgia, Poultry Research Center. Chicks ($n = 25$) were divided equally into 24 floor pens within the facility. Each pen contained nipple drinkers and pan feeders and was setup to simulate commercial broiler house grow-out environments. At placement, necropsy was randomly performed on 5% of the chicks to determine the status of the incoming chicks for the presence of *Salmonella* and *Campylobacter*. At necropsy, the yolk sac and ceca from each bird were aseptically removed, individually placed in sterile sample bags, and transported to the laboratory on ice for evaluation.

For Experiment 1, at 5 wk of age, pens ($n = 12$) were randomly selected and broilers were orally challenged at 10^6 cfu/ml with a characterized strain of *C. jejuni*. The remaining pens of broilers were orally challenged at 10^6 cfu/ml with a cocktail of naladixic acid-resistant *S. enterica* serovar Typhimurium, *S. enterica* serovar Montevideo, and *S. enterica* serovar Heidelburg. For Experiment 2, at 5 wk of age, pens ($n = 12$) were randomly selected and broilers were orally challenged with a 10^5 cfu/ml with a characterized strain of *C. jejuni*.

For each experiment, at 6 wk of age, feed was removed from each pen, and the following morning, the birds from each pen were placed in coops and then processed at the University of Georgia processing facility. The birds were euthanatized, defeathered, and aseptically opened. To prevent contamination of the samples during necropsy, careful measures were taken. The cloaca was externally clamped to prevent fecal spillage from the digestive tract; the outside of each bird was sprayed with 70% ETOH prior to opening of the abdominal cavity (5).

For Experiment 1, necropsy was limited to the removal of unabsorbed yolk (which included free-floating yolk and yolk stalk with yolk attached) and ceca. To reduce the possibility of cross-contamination between tissue samples, the unabsorbed yolk was aseptically removed and then the ceca. Twelve pooled samples (two birds per pool) from the *Salmonella*- and *Campylobacter*-inoculated pens were placed in sterile bags and transported on ice back to the laboratory for assessment. For Experiment 2, necropsy was limited to the removal of the free-floating unabsorbed yolk, yolk stalk, and ceca from each bird ($n = 20$) sampled. Furthermore, to reduce the possibility of cross-contamination between tissues, the unabsorbed yolks (stalk, free, or attached) were aseptically removed, then the ceca. Individual samples were placed in sterile bags and transported on ice to the laboratory for evaluation.

***Campylobacter* lab procedure.** For Experiments 1 and 2, the pooled and individual samples were weighed within plastic bags and then macerated with a rubber mallet to ensure that the contents of the samples were exposed. Bolton's enrichment broth (containing lysed horse blood) was added to the sample bags at a ratio of three times the weight of the sample (volume to weight) and then stomached (Technar Company, Cincinnati, OH) for 1 min. Samples were incubated in a microaerophilic atmosphere at 42 C for 48 hr. A 0.1-ml solution of the enrichment broth from each sample was then duplicate plated onto *Campylobacter* Cefex agar and incubated in microaerophilic atmosphere at 42 C for 48 hr. Following incubation, plates were observed for presumptive *Campylobacter* colonies. Presumptive colonies were confirmed by microscopic observation of characteristic spiral cells and darting motility in wet-mount preparations. Presumptive colonies were then further confirmed using latex agglutination (PANBIO, Inc., Columbia, MD).

***Salmonella* lab procedure.** For Experiments 1 and 2, the unabsorbed yolk and ceca pooled samples were weighed and macerated with a rubber mallet. Next, buffered peptone was added at a ratio of three times the weight of the sample (volume to weight) and stomached for 1 min. The samples were then incubated at 37 C for 24 hr. After incubation, 1/10 ml was then spread plated onto brilliant green sulfa agar containing naladixic acid (200 ppm) in duplicate and incubated for 24 hr at 37 C. Presumptive colonies were then picked and streaked onto lysine iron agar and triple sugar iron slants. Presumptive colonies were then subjected to Poly H and O agglutination and results recorded.

***Enterobacteriaceae* lab procedure for inoculated broilers.** For Experiment 1, serial dilutions were made from the unabsorbed yolk pooled samples prior to the addition of Bolton enrichment broth and spread onto violet red bile glucose agar (VRBG) plates with an overlay in duplicate and incubated at 37 C overnight. Colonies were then counted and data recorded.

Table 1. *Salmonella* and *Campylobacter* positive pooled samples of unabsorbed yolks and ceca from inoculated 6 week old broilers at processing (Experiment 1).

Organisms inoculated	Unabsorbed yolk[A]	Ceca
Campylobacter	9/12[B]	12/12
Salmonella	0/12	2/12

[A]Combination of free-floating yolk and yolk stalk with yolk attached.
[B]Number positive/number sampled.

Aerobic total plate count lab procedure for inoculated broilers. For Experiment 1, serial dilutions were made from the unabsorbed yolk pooled samples prior to the addition of Bolton enrichment broth and spread onto total plate count (TPC) agar plates in duplicate and incubated at 37 C overnight. Colonies were then counted and data recorded.

RESULTS AND DISCUSSION

For Experiments 1 and 2, all chicks sampled prior to placement were negative for the presence of *Campylobacter* and *Salmonella*. For Experiment 1, the *Campylobacter*-inoculated birds had 12/12 pooled ceca and 9/12 unabsorbed pooled yolk samples that were positive for *Campylobacter* (Table 1). The inoculated *Salmonella* were not found in any of the unabsorbed pooled yolk samples and only rarely in the ceca (2/12). This phenomenon has been observed in previous studies (4,5,11). In those previous studies, *Campylobacter* was found to be naturally present in the mature and immature ovarian follicles and in other internal organs, such as the spleen, thymus, and liver/gall-bladder, while *Salmonella* was found at a lower rate. In previous studies and the present study, *Campylobacter* and *Salmonella* tended to differ in the way they established reservoirs in the chicken's body. This could be due to a difference in clearance rates between the two organisms or strain-specific differences, because numerous research studies have shown that, in general, *Campylobacter* is less invasive than *Salmonella*. For the *Campylobacter*-inoculated birds in Experiment 1, the average TPC was $\log_{10}$ 3.5 and the average *Enterobacteriaceae* (ENT) was $\log_{10}$ 3.1 of the pooled samples of unabsorbed yolks. For the *Salmonella*-inoculated birds, the average TPC was $\log_{10}$ 3.4 and the average ENT was 1.9.

In Experiment 2, the samples were not pooled. Samples of the free-floating unabsorbed yolk, yolk stalks with or without attached yolk, and ceca samples were all individually analyzed for the presence of inoculated *Campylobacter* (Table 2). In this experiment, 25% of the broilers had a *Campylobacter*-positive free-floating unabsorbed yolk, while 95% of the broilers sampled had a *Campylobacter*-positive yolk stalk and ceca (Table 2). In the experiment, there was a strong correlation with positive ceca and yolk-stalk samples (18/20 samples).

It has been observed that numerous broilers in the processing plant contain what is commonly refereed to as unabsorbed yolk contents. These yolk contents can be attached to the Meckel's diverticulum or be free floating inside the abdominal cavity of the bird. In this study, it was shown that both inoculated *C. jejuni* and/or *Salmonella* serovars could be detected in these attached or free-floating yolk sacs along with other types of bacteria. The overall significance of this is still to be determined. but this study illustrates that bacteria can live in these unabsorbed yolk contents. The significance of these findings could be that these unabsorbed yolks are a means of recolonization or mechanism of contamination in processing plants.

The yolk stalk is attached to the intestinal tract and the migration of bacteria through the digestive tract would lead one to believe that the bacteria could become trapped in these yolk stalks and contaminate an attached unabsorbed yolk. A contaminated unabsorbed yolk may serve as a reservoir for subsequent pathogen recolonization of the intestine during rearing or contamination of the abdominal cavity if ruptured during processing. Whether or not these yolk stalks have an opening once the yolk becomes detached and free floating is still to be determined.

Table 2. *Campylobacter* positive individual samples of free-floating unabsorbed yolk, yolk stalk, and ceca from *Campylobacter*-inoculated 6-wk-old broilers at processing (experiment 2).

Sample	Free floating	Yolk stalk[A]	Ceca
1	−	+	+
2	−	+	+
3	−	+	+
4	—	+	+
5	+	+	+
6	—	+	−
7	+	+	+
8	+	—	+
9	—	+	+
10	—	+	+
11	—	+	+
12	—	+	+
13	—	+	+
14	—	+	+
15	—	+	+
16	+	+	+
17	—	+	+
18	+	+	+
19	—	+	+
20	—	+	+
Total	5/20	19/20	19/20

[A]Meckel's diverticulum.

In the present study, free-floating unabsorbed yolks were positive for the inoculated organisms, suggesting that the yolk stalks have an opening to the abdominal cavity or that the unabsorbed yolks became unattached and free-floating sometime after inoculation. Additionally, the significance of these unabsorbed yolks as possible reservoirs for these human foodborne enteropathogens in broilers and possible modes of contamination in the processing plant is yet to be determined. The next set of experiments will determine the natural incidence of these unabsorbed yolks in different age commercial broilers. The natural occurrence of *Campylobacter*, *Salmonella*, and the types of total bacteria and *Enterobacteriaceae* found in these tissues will also be investigated. Further research needs to be conducted to determine the significance of these findings from a recolonization and processing-contamination stand point.

REFERENCES

1. Anthony, N. B., E. Dunnington, and P. B. Siegel. Embryo growth of the normal and dwarf chickens from lines selected for high and low 56 d body weight. Arch. Geflugeldk. 53:116–122. 1989.

2. Bailey, J. S., N. A. Cox, D. E. Cosby, and L. J. Richardson. Movement and persistence of *Salmonella* in broiler chicken following oral or intracloacal inoculation. J. Food Prot. 68:2698–2701. 2005.

3. Chamblee, T. N., J. D. Brake, C. D. Schultz, and J. P. Thaxton. Yolk sac absorption and initiation of growth in broilers. Poult. Sci. 71: 1811–1816. 1992.

4. Cox, N. A., J. S. Bailey, L. J. Richardson, R. J. Buhr, D. E. Cosby, J. L. Wilson, K. L. Hiett, G. R. Siragusa, and D. V. Bourassa. Presence of naturally occurring *Campylobacter* and *Salmonella* in the mature and immature ovarian follicles of late-life broiler breeder hens. Avian Dis. 49: 285–287. 2005.

5. Cox, N. A., C. L. Hofacre, J. S. Bailey, R. J. Buhr, J. L. Wilson, D. E. Cosby, M. T. Musgrove, L. J. Richardson, J. D. Tankson, Y. L. Vizzier, P. F. Cray, K. L. Hiett, L. E. Vaughn, P. S. Holt, and D. V. Bourassa. Presence of *Campylobacter jejuni* in various organs one hour, one day, and one week following oral or intracloacal inoculations of broiler chicks. Avian Dis. 49: 155–158. 2005.

6. Esteban, S., M. Noreno, J. M. Rayo, and J. A. Tur. Gastrointestinal emptying in the final days of incubation of the chick embryo. Poult. Sci. 32:279–284. 1991.

7. Friedman, C. R., J. Neimann, H. C. Wegener, and R. V. Tauxe. Epidemiology of *C. jejuni* infections in the United States and other industrialized nations. In: Campylobacter, 2nd ed. I. Nachamkin, I. and M. J. Blaser, eds. ASM Press, Washington, DC. pp. 121–138. 2000.

8. Grant, I. H., N. J. Richardson, and V. D. Bokkenheuser. Broiler chickens as potential source of *Campylobacter* infections in humans. J. Clin. Microbiol. 11:508–510. 1980.

9. Jacobs-Reitsma, W. F. *Campylobacter* in the food supply. In: Campylobacter, 2nd ed. I. Nachamkin, I. and M. J. Blaser, eds. ASM Press, Washington, DC. pp. 467–481. 2000.

10. Jin, S. H., A. Corless, and J. Sell. Digestive system development in post-hatch poultry. World Poult. Sci. J. 57:197–205. 1998.

11. Jones, F. T., R. C. Axtell, d. V. Rives, S. E. Scheideler, F. R. Tarver, R. L. Walker, and M. J. Wineland. A survey of *Salmonella* contamination in modern broiler production. Int. Ass. Milk Food Env. Sanitarians 54: 502–507. 1991.

12. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607–625. 1999.

13. Noy, Y., and D. Sklan. Digestion and absorption in the young chick. Poult. Sci. 74:366–373. 1995.

14. Noy, Y., and D. Sklan. Utilization of yolk in the newly hatched poult. Br. Poult. Sci. 39:446–451. 1998.

15. Noy, Y., and D. Sklan. Energy utilization in newly hatched chicks. Poult. Sci. 78:1750–1756. 1999.

16. Noy, Y., Z. Uni, and D. Sklan. Routes of yolk utilization in the newly-hatched chick. Poult. Sci. 37:987–996. 1996.

17. Olsen, S. J., L. C. MacKinnon, J. S. Goulding, N. H. Bean, and L. Slutsker. Surveillance for foodborne disease outbreaks—United States, 1993–1997. Morb. Mortal. Wkly. Rep. 49:1–62. 2000.

18. Oosterom, J., S. Notermans, H. Karman, and G. B. Engels. Origin and prevalence of *Campylobacter jejuni* in poultry processing. J. Food Prot. 46:339–344. 1983.

19. Romanoff, A. L. The avian embryo. Macmillan, New York. pp. 1042–1081. 1960.

20. Roy, P., A. S. Dhillon, L. H. Lauerman, D. M. Schaberg, D. Bandli, and S. Johnson. Results of Salmonella isolation from poultry products, poultry, poultry environment, and other characteristics. Avian Dis. 46:17–24. 2002.

21. Simmons, M., D. L. Fletcher, J. A. Cason, and M. E. Berrang. Recovery of *Salmonella* from retail broilers by a whole-carcass enrichment procedure. J. Food Prot. 66:446–450. 2003.

22. Solomon, E. B., and D. G. Hoover. *Campylobacter jejuni*: a bacterial paradox. J. Food Safety 19:121–136. 1999.

23. Zhao, T., G. O. I. Ezeike, M. P. Doyle, Y. Hung, and R. S. Howell. Reduction of *Campylobacter jejuni* on poultry by low-temperature treatment. J. Food Prot. 66:652–655. 2003.